

## EVALUATION AND COMPARISON OF RAPID CARD TEST WITH IgM ELISA FOR DIAGNOSIS OF SCRUB TYPHUS: A STUDY DONE IN PERIPHERAL ZONAL HOSPITAL OF HIMACHAL PRADESH (INDIA)

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**ABSTRACT: Background:** Scrub typhus is a tropical febrile zoonotic disease caused by *Orientia tsutsugamushi* of the *rickettsial* family. Clinical diagnosis of scrub typhus is often difficult because of similarity of symptoms to other febrile illnesses such as dengue, leptospirosis, malaria and other viral hemorrhagic fevers. Accuracy and rapidity of a diagnostic test to *Orientia tsutsugamushi* is an important step to diagnose this disease. **Method:** The present study was performed to compare the results of recently introduced IgM ELISA, considered to be a reference standard and rapid card test Bioline SD *tsutsugamushi* on 232 non-repeated serum samples of patients, suspected to be suffering from Scrub Typhus. **Results:** Out of 232 patients, 32 (13.8%) patients were found to be serologically positive for Scrub Typhus by IgM ELISA and 30 (12.9%) showed positive results by rapid card test Bioline SD *tsutsugamushi*. Maximum patients (75%) were between the age from 20-50 years. Majority i.e 17 (53.1%) of patients were females. Sensitivity and Specificity of Rapid card test in relation to IgM ELISA was found to be 93.75% and 100% respectively. Positive Predictive Value of Rapid Card test was found to be 100% and Negative Predictive Value was found to be 99.04%. **Conclusion:** The rapid Scrub Typhus IgM kit can be used as a Point-of-Care (PoC) test as it showed good sensitivity and specificity. The kit would be helpful in both urban and remote rural parts of Himachal Pradesh for the rapid diagnosis of Scrub typhus.

**KEY WORDS:** Scrub typhus, Rapid Card Test, IgM ELISA

### INTRODUCTION:

Scrub typhus is a zoonotic acute febrile illness caused by *Orientia tsutsugamushi* of

the *rickettsial* family. It is an emerging infectious disease of India and is now reported from almost

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every state.<sup>1-6</sup> It is transmitted to humans by larvae (or chiggers) of several species of trombiculid mites. Scrub typhus was originally thought to be confined to jungles, but now it is prevalent in both rural and urban areas. This might be perhaps due to the migration of people and clearing of forests for building houses, factories etc. The 'tsutsugamushi triangle' is now slowly progressing to other continents – Africa, Europe and South America.<sup>7,8-10</sup>

The disease poses a serious threat to public health if not diagnosed properly. Diagnosis of scrub typhus is still a challenge to both clinical and laboratory people. Generally the diagnosis of scrub typhus is based on the patient's clinical presentation and history. Presence of an eschar and history of travel to an endemic area often help in clinching the diagnosis.<sup>11</sup> Accurate diagnosis leads to proper treatment and thus preventing lethal complications.<sup>12</sup>

Laboratory diagnosis of the disease is mainly based on serological and molecular assays. Cultures of *O. tsutsugamushi* need dedicated laboratory facilities along with expertise. The Weil-Felix test is the still most commonly used test for diagnosis of scrub typhus; however, it lacks both sensitivity and specificity.<sup>13,14</sup> Recently methods such as a latex agglutination test and rapid immunochromatographic flow assay have become popular because of their short turnaround times and ease of performance; however, they require validation before being used for routine diagnosis.<sup>15,16</sup> Thus research in Scrub typhus has been facilitated by the availability of specific rapid Point-of-Care test (PoC) and other conventional serological tests like ELISA, Indirect Immunofluorescence Assay (IFA) and molecular diagnostic test targeting *Orientia tsutsugamushi* DNA. Himachal Pradesh is an endemic state for Scrub Typhus. Various serological tests are available for the diagnosis, of which the Weil Felix Test (WFT) is most widely used test.

Recently, IgM ELISA for Scrub Typhus has been introduced in our set up of Zonal Hospital for the diagnosis of Scrub Typhus. Before this, we were using highly sensitive and specific rapid card test named Bioline SD Tsutsugamushi for diagnosis of Scrub typhus in our peripheral set up of Zonal Hospital. The present study was done to establish the diagnosis of scrub typhus by IgM ELISA and to compare rapid card test with IgM ELISA in the diagnosis of Scrub Typhus.

## **MATERIALS AND METHODS:**

The study was conducted in the Microbiology Section of Laboratory of Zonal Hospital Mandi a peripheral institute of Himachal Pradesh. It was a prospective cross-sectional study of six months duration starting from September 2016 to February 2017. The study group included clinically suspected patients of Scrub Typhus. Inclusion Criteria was patients having signs and symptoms correlating to Scrub Typhus like fever, headache, myalgia, abdominal pain, altered sensorium, respiratory distress, jaundice, hepatosplenomegaly, lymphadenopathy, eschar and rash. Patients who were suffering from Dengue, Malaria and other causes of PUO were excluded from the study group.

Taking all aseptic precautions five milliliter of venous blood was collected from each patient and was centrifuged at 2500 rpm for 4-5 minutes to separate serum. Each separated sera was stored in screw capped vial at 4°C till the tests were performed. This sera was processed for IgM ELISA for Scrub Typhus and Rapid Card Test as follows:

(1) IgM InBios ELISA: IgM ELISA kits manufactured by In Bios International Inc. were used. The ELISA plates with 96 wells were coated with ten recombinant antigens of *O. tsutsugamushi*, targeting antibodies to the 56-kDa antigen. The test was performed as per manufacturer's protocol. OD (Optical Density) readings were taken at 450nm in iMark Microplate

Reader (Bio-Rad, Japan). Cut-off values were calculated and interpretation of the test results was computed. Briefly, 20 samples from healthy volunteers from ST endemic area were tested by IgM InBios ELISA and average OD value was taken as cut-off value. Cut-off values were calculated as follows:

Cut-off value = Average of the Normal Human Sera (NHS) + three times SD of NHS. The samples with OD values above the Cut-off (0.560) were considered positive and those below the Cut-off were taken as negative.

(2) SD BiolineTsutsugamushi (One Step Scrub Typhus Antibody Test): The test was performed in accordance to the technical brochure provided with the kit. Briefly, 10 $\mu$ l of serum sample was added to the specimen well of the test device, followed by the addition of 3-4 drops (100-200  $\mu$ l) of assay diluent provided in the kit. Results were read within 10-15 minutes. A single red line appears on the control area and if the patient has Scrub typhus antibody, a second red line appeared on the test area.

Statistical Analysis Data collected was statistically analyzed using Open Epi Info Version 3, open source calculator-Diagnostic Test.

## **RESULTS:**

The study was conducted on serum samples of 232 patients, suspected to be suffering from Scrub Typhus. . IgM ELISA for Scrub Typhus was positive in 13.8% (32/232) patients and Rapid Card test was positive in 12.9% (30/232) of the patients. Maximum patients were between the age from 20-50 years. Majority (53.1%) of patients were females. Out of these 32 (13.8%) patients were found to be serologically positive for Scrub Typhus by IgM ELISA and 30 (12.9%) showed positive results by rapid card test Bioline SD Tsutsugamushi. All the 30 samples tested

positive by rapid card test were also positive by IgM ELISA for scrub typhus. Only two patients who were positive by IgM ELISA were found to be negative by rapid card test. Sensitivity and Specificity of Rapid card test in relation to IgM ELISA was found to be 93.75% and 100% respectively. Positive Predictive value of Rapid Card test was found to be 100% and Negative Predictive Value was found to be 99.04%.

## **DISCUSSION:**

The present study was carried out to evaluate and compare Rapid Card test in relation to IgM ELISA, taken as reference standard for the diagnosis of Scrub. IgM ELISA was earlier validated by several researchers in India and abroad with satisfactory performance, qualifying as an alternate reference test to IFA Typhus patients.<sup>4-6,17-20,21</sup> Various Rapid Kits although available in Indian Market, has not been validated in our set up so far, although available in Indian market for the past one year, has not been validated so far. Hence, we present our findings validating rapid kit SD BiolineTsutsugamushi for Scrub typhus sero-diagnosis with sensitivity and specificity of 93.75% and 100% respectively. Positive predictive value of Rapid Card test was found to be 100% and Negative Predictive Value was found to be 99.04%. Kingston et al., from Thailand, validated ST InBios Rapid kit (ICT) kit against the gold standard IFA and reported a satisfactory performance of 92% sensitivity and 95% specificity.<sup>20</sup> ImmuneMed rapid kit is has been validated by Korean researchers and found to have 98.6% sensitivity and 98.2% specificity.<sup>22</sup> In a study by Velmurugan A et al, ImmuneMed rapid kit performed equally well with 94.87%, sensitivity and 94.19% specificity.<sup>23</sup> IgM ELISA for Scrub Typhus was positive in 13.8% (32/232) patients. The finding has similarity to the studies done by P. Sinha et al and KPS Narvencar et al who reported 24.7% and 34%

positivity in their respective studies.<sup>24,25</sup> A. Mahajan et al in an outbreak in 2009 in Jammu reported 74% patients positive by IgM ELISA for Scrub Typhus which was very much higher than our study.<sup>26</sup>

In the study the majority of the patients were in the age group between 20-50 years. The factor is being attributed to the active years of life, when people are mostly involved in outdoor activities. Our finding is in concordance to the study of PK Sharma et al.<sup>27</sup> Majority 17 (53.1%) of patients were females and 15 (46.7%) with a male: female ratio of 1:1.06. Females are more affected than males due to their more involvement in fields and thus exposure to vegetation's. Our findings were similar to the findings in the studies done by PK Sharma et al and SS Kweon et al which showed female preponderance of 54.8% and 64.5% respectively.<sup>27,28</sup> All the patients present in the study were from rural background which goes well with other studies.

In our study the twenty nine out of thirty two (90.6%) patients reported in the month of September to October. The surge of cases corresponded to the monsoon season of our area in which growth of scrub and other vegetations are at their peak. This type of seasonal trend was also seen by MVS Subhalaxmi et al and in their studies where the confirmed cases peaked in autumn.<sup>29</sup>

## **CONCLUSION:**

Although IgM ELISA for Scrub Typhus is more sensitive, rapid and specific for testing large number of sera in early phase of disease, but considering the high specificity (100%) and less technicality, the rapid card test is a good option as Point-of-Care (PoC) test. We interpret from our study, that Himachal Pradesh being an endemic state, Rapid Diagnostic test kits should be in place in hospitals and other health centers owing to its accuracy, rapidity, simplicity, and low necessity for skill to improve the rapid detection of scrub typhus. Further research is recommended in field.

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